on STN

ACCESSION NUMBER: 87058717 EMBASE
DOCUMENT NUMBER: 1987058717
TITLE: In vitro packaging into phage T4 particles and specific recircularization of phage lambda DNAs.
AUTHOR: Black L.W.
CORPORATE SOURCE: Department of Biochemistry, University of Maryland Medical School, Baltimore, MD 21201, United States
SOURCE: Gene, (1986) 46/1 (97-101).
CODEN: GENED6
COUNTRY: Netherlands
DOCUMENT TYPE: Journal
FILE SEGMENT: 004 Microbiology
022 Human Genetics
047 Virology
LANGUAGE: English
AB Concatemeric phage λimm434 DNA packaged in vitro into phage T4 particles produced plaques on a selective host. Moreover, λ DNA containing a pBR322 derivative flanked by the λ attL and **attR** sites could be specifically recircularized by excisive λ **recombination** to yield the pBR322 derivative. A host deficient in generalized **recombination** and containing a defective λcIts prophage which provided Int and Xis proteins was the recipient for this **plasmid** derivative carried by T4. Such a T4-λ **hybrid** may potentially allow almost one T4 headful of donor DNA (166 kb) to be packaged and recircularized.

NSWER 1 OF 22 MEDLINE on STN
ACCESSION NUMBER: 1999377766 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10448644
TITLE: Efficient generation of recombinant adenoviral vectors by Cre-**lox recombination** in vitro.
AUTHOR: Aoki K; Barker C; Danthinne X; Imperiale M J; Nabel G J
CORPORATE SOURCE: Howard Hughes Medical Institute, University of Michigan Medical Center, Department of Internal Medicine and Biological Chemistry, Ann Arbor 48109-0650, USA.
CONTRACT NUMBER: GM34902 (NIGMS)
SOURCE: Molecular medicine (Cambridge, Mass.), (1999 Apr) 5 (4) 224-31.
Journal code: 9501023. ISSN: 1076-1551.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990925
Last Updated on STN: 19990925
Entered Medline: 19990913

AB BACKGROUND: Although recombinant adenovirus vectors are attractive for use in gene expression studies and therapeutic applications, the construction of these vectors remains relatively time-consuming. We report here a strategy that simplifies the production of adenoviruses using the Cre-**loxP** system. MATERIALS AND METHODS: Full-length recombinant adenovirus DNA was generated in vitro by Cre-mediated **recombination** between **loxP** sites in a linearized **shuttle plasmid** containing a transgene and adenovirus genomic DNA. RESULTS: After transfection of Cre-treated DNA into 293 cells, replication-defective viral vectors were rapidly obtained without detectable wild-type virus. CONCLUSION: This system facilitates the development of recombinant adenoviral vectors for basic and clinical research.

L16 ANSWER 2 OF 22 MEDLINE on STN
ACCESSION NUMBER: 97184512 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9032314
TITLE: Construction of adenovirus vectors through Cre-**lox recombination**.
AUTHOR: Hardy S; Kitamura M; Harris-Stansil T; Dai Y; Phipps M L
CORPORATE SOURCE: Somatix Therapy Corporation, Alameda, California 94501-1034, USA.. shardy@apple.somatix.com
SOURCE: Journal of virology, (1997 Mar) 71 (3) 1842-9.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U62024
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970327
Last Updated on STN: 19970327
Entered Medline: 19970318

AB Two barriers prevent adenovirus-based vectors from having wide application. One is the difficulty of making new adenoviruses, and the second is the strong immunological reaction to viral proteins. Here we describe uses of Cre-**lox recombination** to overcome these problems. First, we demonstrate a simple method for constructing E1-substituted adenoviruses. Second, we demonstrate a method to construct adenovirus vectors carrying recombinant genes in place of all of the viral genes, so-called gutless adenovirus vectors. The pivotal feature in each method is the use of a negatively selected adenovirus named psi5. We

engineered a cis-acting selection into psi5 by flanking its packaging site with **loxP** sites. When psi5 was grown in cells making a high level of Cre **recombinase**, the packaging site was deleted by **recombination** and the yield of psi5 was reduced to 5% of the wild-type level. To make a new E1-substituted virus, we used psi5 as a donor virus and recombined it with a **shuttle vector** via a **loxP** site. The resulting recombinant virus has a single **loxP** site next to the packaging site and therefore outgrows psi5 in the presence of Cre **recombinase**. To make a gutless virus, we used psi5 as a helper virus. The only viral sequences included in the gutless vector are those needed in cis for its replication and packaging. We found that a **loxP** site next to the packaging site of the gutless virus was necessary to neutralize homologous **recombination** between psi5 and the gutless viruses within their packaging domains.

L16 ANSWER 3 OF 22 MEDLINE on STN
ACCESSION NUMBER: 97105907 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8948655
TITLE: Rapid construction in yeast of complex targeting vectors for gene manipulation in the mouse.
AUTHOR: Storck T; Kruth U; Kolhekar R; Sprengel R; Seeburg P H
CORPORATE SOURCE: Center for Molecular Biology (ZMBH), University of Heidelberg, Germany.. storck@sun0.urz.uni-heidelberg.de
SOURCE: Nucleic acids research, (1996 Nov 15) 24 (22) 4594-6. Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U63018; GENBANK-U63120
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970117

AB Targeting vectors for embryonic stem (ES) cells typically contain a mouse gene segment of >7 kb with the neo gene inserted for positive selection of the targeting event. More complex targeting vectors carry additional genetic elements (e.g. lacZ, **loxP**, point mutations). Here we use homologous **recombination** in yeast to construct targeting vectors for the incorporation of genetic elements (GEs) into mouse genes. The precise insertion of GEs into any position of a mouse gene segment cloned in an Escherichia coli/yeast **shuttle vector** is directed by short recombinogenic arms (RAs) flanking the GEs. In this way, complex targeting vectors can be engineered with considerable ease and speed, obviating extensive gene mapping in search for suitable restriction sites.

L16 ANSWER 4 OF 22 MEDLINE on STN
ACCESSION NUMBER: 96106831 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7503713
TITLE: Efficient regulation of gene expression by adenovirus vector-mediated delivery of the CRE **recombinase**.
AUTHOR: Sakai K; Mitani K; Miyazaki J
CORPORATE SOURCE: Department of Disease-related Gene Regulation Research (Sandoz), University of Tokyo, Japan.
SOURCE: Biochemical and biophysical research communications, (1995 Dec 14) 217 (2) 393-401. Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601

ENTRY DATE: Entered STN: 19960217
Last Updated on STN: 19960217
Entered Medline: 19960118

AB We have constructed an E1-defective adenovirus (Ad) **vector** designated AdCAG-Cre containing the Cre **recombinase** gene derived from bacteriophage P1 under control of the cytomegalovirus immediate early enhancer-chicken beta-actin **hybrid** (CAG) promoter. We examined the Cre-**loxP**-based **recombination** by this Ad vector in C2C12 cells bearing a reporter gene construct CAG-CAT-Z, which directs expression of the E. coli lacZ gene upon Cre-mediated excision of the CAT gene located between the CAG promoter and the lacZ gene. Nearly 100% of these cells were shown to start to produce beta-galactosidase after infection with the AdCAG-Cre vector at MOI 100. On the basis of this result, we discuss the possible use of the AdCAG-Cre vector to manipulate the gene expression in mammalian cells.

L16 ANSWER 5 OF 22 MEDLINE on STN
ACCESSION NUMBER: 95291341 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7773312
TITLE: A novel Ti-plasmid-convertible lambda phage vector system suitable for gene isolation by genetic complementation of Arabidopsis thaliana mutants.
AUTHOR: Fuse T; Kodama H; Hayashida N; Shinozaki K; Nishimura M; Iba K
CORPORATE SOURCE: Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan.
SOURCE: Plant journal : for cell and molecular biology, (1995 May) 7 (5) 849-56.
Journal code: 9207397. ISSN: 0960-7412.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950720
Last Updated on STN: 19950720
Entered Medline: 19950713

AB A new lambda phage vector system, lambda TI, has been constructed to facilitate genetic complementation of higher plant mutations. The lambda TI vectors are stable, and by using the Cre-**lox** site-specific **recombination**, are automatically convertible into Ti-**plasmid binary** vectors which are capable of expressing genes in higher plants. Two lambda TI vectors were constructed: (i) lambda TI1, which can generate a Ti-plasmid that contains the cauliflower mosaic virus (CaMV) 35S promoter and is suitable for the expression of cDNA in transformed plants and (ii) lambda TI2, which can generate a Ti-plasmid with the multicloning site (MCS). cDNA and genomic libraries, which were constructed from the cruciferous plant Arabidopsis thaliana in these lambda TI vectors, can be probed by large DNA fragments of more than 100 kb, such as yeast artificial chromosomes (YACs), enabling the direct screening of the clones in the chromosome region containing a specified genetic locus. These libraries will certainly become powerful tools for the genetic complementation of Arabidopsis mutant phenotypes by quickly providing transformation-competent clones.

L16 ANSWER 6 OF 22 MEDLINE on STN
ACCESSION NUMBER: 94355044 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8074871
TITLE: Lambda/plasmid vector construction by in vivo cre/**lox**-mediated **recombination**.
AUTHOR: Brunelli J P; Pall M L
CORPORATE SOURCE: Washington State University, Pullman 99164-4234.
SOURCE: BioTechniques, (1994 Jun) 16 (6) 1060-4.

Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
DOCUMENT TYPE: Report; (TECHNICAL REPORT)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941013
Last Updated on STN: 19941013
Entered Medline: 19941006

AB Lambda/**plasmid** **hybrid** vectors have been previously constructed in which the **plasmid** sequences are separated from flanking lambda arms by **lox** sites. The **lox** sequence is the substrate of Cre-mediated site-specific **recombination**, allowing easy excision of plasmid sequences (automatic subcloning). We have developed a simple procedure to construct other such lambda **hybrid** vectors using in vivo cre/**lox**-mediated **recombination** to exchange new plasmids for plasmids previously incorporated into lambda/**plasmid** hybrids. Because **hybrid** vectors both with and without lacZ alpha **plasmid** sequences are available, producing either blue or clear plaques, respectively, the new lambda **hybrid** vectors can be distinguished from the parental hybrids by blue/clear plaque screening. This procedure has been successfully used to construct ten hybrid vectors. It generates new lambda/**plasmid** **hybrid** vectors, without ligation or lambda packaging, which retain the property of automatic subcloning.

L16 ANSWER 7 OF 22 MEDLINE on STN
ACCESSION NUMBER: 92235813 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1569546
TITLE: The mcm2 mutation of yeast affects replication, rather than segregation or amplification of the two micron plasmid.
AUTHOR: Maiti A K; Sinha P
CORPORATE SOURCE: Department of Biochemistry, Bose Institute, Calcutta, India.
SOURCE: Journal of molecular biology, (1992 Apr 5) 224 (3) 545-58.
Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 19920612
Last Updated on STN: 19920612
Entered Medline: 19920526

AB We have studied the maintenance of the endogenous two micron (2 mu) plasmid in a strain of yeast carrying the nuclear mutation mcm2. This mutation, earlier shown to affect the maintenance of yeast minichromosomes in an ARS-dependent manner, also affected the copy number of the 2 mu plasmid. The effect was more pronounced at 35 degrees C leading to the elimination of the plasmid from the cells cultured at this temperature. The mutant cells could be efficiently cured of the circle by transformation with 2 mu ORI-carrying **hybrid** vectors, an observation consistent with the low copy number of the endogenous **plasmid**. A chromosomal revertant of this mutant for another ARS(ARS1) was found also to confer stability on the 2 mu ORI-carrying minichromosomes and had elevated levels of the endogenous plasmid. The mutation neither affected the segregation nor the amplification process mediated by site-specific **recombination** at **FRT** sites requiring the FLP gene-encoded protein action. ARS131C, an ARS that was unaffected in the mutant at 25 degrees C, could elevate the copy number of a 2 mu **hybrid** **vector** in the mutant cells. In view of these results, some aspects of segregation and copy number control of the endogeneous plasmid have been discussed. We propose that the mutation

impairs the 2 mu ORI function, leading to its loss.

L16 ANSWER 8 OF 22 MEDLINE on STN
ACCESSION NUMBER: 90147639 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2695072
TITLE: REP3-derived yeast **shuttle vector**.
AUTHOR: Schubert U; Gniel D; Lang H
CORPORATE SOURCE: Central Institute of Microbiology and Experimental Therapy, Academy of Sciences of the GDR, Jena.
SOURCE: Biomedica biochimica acta, (1989) 48 (8) 529-37. Journal code: 8304435. ISSN: 0232-766X.
PUB. COUNTRY: GERMANY, EAST: German Democratic Republic
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199002
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19900226

AB A yeast-**shuttle vector** using a XbaI-PstI fragment of the 2 microns DNA 1293 bp in length has been constructed. This sequence spans the REP 3 locus and the origin of replication. Besides the 2 microns DNA derived sequences the resulting yeast **shuttle vector** contains the yeast LEU 2 gene and the Tet' gene of pBR 322. The results demonstrate that the XbaI-PstI fragment is sufficient for proper amplification and partitioning of 2 microns DNA derived yeast-shuttle vectors in yeast. The interruption of the **FRT** site in this type of **vector** seems to prevent any **recombination** between endogenous 2 microns DNA and **hybrid plasmid** molecules. Moreover, the disturbance of the FLP-system has no influence on the copy number of the plasmid stability.

L16 ANSWER 9 OF 22 MEDLINE on STN
ACCESSION NUMBER: 88314876 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3045079
TITLE: Characterization of the rec-1 gene of Haemophilus influenzae and behavior of the gene in Escherichia coli.
AUTHOR: Setlow J K; Spikes D; Griffin K
CORPORATE SOURCE: Biology Department, Brookhaven National Laboratory, Upton, New York 11973.
SOURCE: Journal of bacteriology, (1988 Sep) 170 (9) 3876-81. Journal code: 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198810
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19881006

AB The rec-1 gene of Haemophilus influenzae was cloned into a **shuttle vector** that replicates in Escherichia coli as well as in H. influenzae. The plasmid, called pRec1, complemented the defects of a rec-1 mutant in repair of UV damage, transformation, and ability of prophage to be induced by UV radiation. Although UV resistance and **recombination** were caused by pRec1 in E. coli recA mutants, UV induction of lambda and UV mutagenesis were not. We suggest that the ability of the H. influenzae Rec-1 protein to cause cleavage of repressors but not the **recombinase** function differs from that of the E. coli RecA protein.

L16 ANSWER 10 OF 22 MEDLINE on STN
ACCESSION NUMBER: 87089725 MEDLINE

DOCUMENT NUMBER: PubMed ID: 3025614
TITLE: Mating type-like conversion promoted by the 2 micrograms circle site-specific **recombinase**: implications for the double-strand-gap repair model.
AUTHOR: Jayaram M
SOURCE: Molecular and cellular biology, (1986 Nov) 6 (11) 3831-7. Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198702
ENTRY DATE: Entered STN: 19900302
Last Updated on STN: 19990129
Entered Medline: 19870217

AB Double-strand breaks in DNA are known to promote **recombination** in Saccharomyces cerevisiae. Yeast mating type switching, which is a highly efficient gene conversion event, is apparently initiated by a site-specific double-strand break. The 2 micrograms circle site-specific **recombinase**, FLP, has been shown to make double-strand breaks in its substrate DNA. By using a **hybrid** 2 micrograms circle::Tn5 **plasmid**, a portion of which resembles, in its DNA organization, the active (MAT) and the silent (HML) yeast mating type loci, it is shown that FLP mediates a conversion event analogous to mating type switching. Whereas the FLP site-specific **recombination** is not dependent on the RAD52 gene product, the FLP-induced conversion is abolished in a rad52 background. The FLP-promoted conversion in vivo can be faithfully reproduced by making a double-stranded gap in vitro in the vicinity of the FLP site and allowing the gap to be repaired in vivo.

L16 ANSWER 11 OF 22 MEDLINE on STN
ACCESSION NUMBER: 81237782 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6265316
TITLE: Functional analysis of hybrid plasmids carrying genes for lambda site-specific **recombination**.
AUTHOR: Strizhov N; Soukovatitsin V; Ksenzenko V; Tikhomirova L; Bayev A
SOURCE: Gene, (1980 Dec) 12 (3-4) 201-14. Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198109
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19900316
Entered Medline: 19810915

AB A number of hybrid plasmids, carrying lambda genes involved in site-specific integrative **recombination**, have been constructed in vitro. Analysis of protein synthesis in Escherichia coli minicells has shown that Int protein is synthesized only when int gene is expressed constitutively. The plasmids RSF2124::lambda-CD, RSF2124::lambda-Cint-c57, and pInt lambda were able to integrate into the chromosome of E.coli at the **attB**. The integration of hybrid plasmids into the genome of bacteria has also been shown for polA1 strains restricting the autonomous replication of ColE1 type plasmids. Genetic markers of hybrid plasmids are maintained in polA1 bacteria for at least 50 generations under nonselective conditions. The Southern blotting experiments using [32P]pBR322 DNA and EcoRI fragments of E. coli polA1 chromosome carrying integrated **plasmid** pInt lambda demonstrated that in this strain **hybrid** plasmids can be observed only when integrated into the **attB** of the chromosome according to Campbell's model of integration. In the cells, where autonomous replication of plasmids is

possible, they can be observed both in extrachromosomal and integrated states. The integration of the ColE1 replication origin into the chromosome of bacteria is not lethal for the cells. Only **attP** and the int gene of lambda are necessary for the integration of hybrid plasmids under conditions of effective int gene expression. If the level of Int protein synthesis is high enough, the prophage excision can be observed in the absence of Xis product. The six-fold decrease of Int protein concentration in the cell (in case of pInt lambda 2 as compared to pInt lambda 1) is critical both for integration and excision.

L16 ANSWER 12 OF 22 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 1999246117 EMBASE
TITLE: Reversible immortalization of human myogenic cells by site-specific excision of a retrovitally transferred oncogene.
AUTHOR: Berghella L.; De Angelis L.; Coletta M.; Berarducci B.; Sonnino C.; Salvatori G.; Anthonissen C.; Cooper R.; Butler-Browne G.S.; Mouly V.; Ferrari G.; Mavilio F.; Cossu G.
CORPORATE SOURCE: Dr. G. Cossu, Dept. of Histol. and Med. Embryol., University of Roma La Sapienza, Via A. Scarpa 14, 00161 Rome, Italy. cossu@axrma.uniromal.it
SOURCE: Human Gene Therapy, (1 Jul 1999) 10/10 (1607-1617). Refs: 41
ISSN: 1043-0342 CODEN: HGTHE3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Myogenic cells have a limited life span in culture, which prevents expansion at clinically relevant levels, and seriously limits any potential use in cell replacement or ex vivo gene therapy. We developed a strategy for reversibly immortalizing human primary myogenic cells, based on retrovirus- mediated integration of a wild-type SV40 large-T antigen (Tag), excisable by means of the Cre-**Lox recombination** system. Myogenic cells were transduced with a **vector** (LTTN-**LoxP**) expressing the SV40 Tag under the control of an LTR modified by the insertion of a **LoxP** site in the U3 region. Clonal isolates of Tag-positive cells showed modified growth characteristics and a significantly extended life span, while maintaining a full myogenic potential. Transient expression of Cre **recombinase**, delivered by transfection or adenoviral **vector** transduction, allowed excision of the entire provirus with up to >90% efficiency. Cultures of Cre-treated (Tag-) or untreated (Tag+) myogenic cells were genetically labeled with a lacZ retroviral **vector**, and injected into the regenerating muscle of SCID/bg immunodeficient mice. Tag- cells underwent terminal differentiation in vivo, giving rise to clusters of β- Gal+ **hybrid** fibers with an efficiency comparable to that of control untransduced cells. Tag+ cells could not be detected after injection. Neither Tag+ nor Tag- cells formed tumor in this xenotransplantation model. Reversible immortalization by Tag therefore allows the expansion of primary myogenic cells in culture without compromising their ability to differentiate in vivo, and could represent a safe method by which to increase the availability of these cells for clinical application.

L16 ANSWER 13 OF 22 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 96150435 EMBASE
DOCUMENT NUMBER: 1996150435
TITLE: Accessory proteins impose site selectivity during ColE1

dimer resolution.
AUTHOR: Guhathakurta A.; Viney I.; Summers D.
CORPORATE SOURCE: Department of Genetics, Downing Street,Cambridge CB2 3EH, United Kingdom
SOURCE: Molecular Microbiology, (1996) 20/3 (613-620).
ISSN: 0950-382X CODEN: MOMIEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The cer-Xer dimer resolution system of **plasmid** ColE1 is highly selective, acting only at sites on the same molecule and in direct repeat. **Recombination** requires the XerCD **recombinase** and accessory proteins ArgR and pepA. The Escherichia coli chromosome dimer resolution site dif and the type II **hybrid** site use the same **recombinase** but are independent of ArgR and PepA and show no site selectivity. This has led to the proposal that ArgR and PepA are responsible for the imposition of constraint. We describe here the characterization of a novel class of 'conditionally constrained' multimer resolution sites whose properties support this hypothesis. In the presence of ArgR and PepA, plasmids containing conditionally constrained sites are monomeric, but in their absence, extensive multimerisation is seen. A mutant ArgR derivative (ArgR110), which is defective in cer-mediated dimer resolution, remains able to prevent **plasmid** multimerisation by a conditionally constrained site. This implies that the accessory factors block **recombination** in trans rather than ensuring rapid multimer resolution. When the distance between the ArgR and XerCD binding sites in a conditionally constrained site was altered by a non-integral number of helical turns, the site became unconstrained. Constraint was restored by the insertion of a full helical turn.

L16 ANSWER 14 OF 22 EMBASE
on STN
ACCESSION NUMBER: 95175546 EMBASE
DOCUMENT NUMBER: 1995175546
TITLE: Involvement of ArgR and PepA in the pairing of ColE1 dimer resolution sites.
AUTHOR: Guhathakurta A.; Summers D.
CORPORATE SOURCE: Department of Genetics, Downing Street,Cambridge CBZ 3EH, United Kingdom
SOURCE: Microbiology, (1995) 141/5 (1163-1171).
ISSN: 1350-0872 CODEN: MROBEO
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Dimer formation and associated copy number depression is an important cause of multicopy **plasmid** instability. Natural multicopy plasmids employ site-specific **recombination** to convert dimers to monomers, thus maximizing the number of independently segregating molecules at cell division. Resolution of dimers of Escherichia coli **plasmid** ColE1 requires the **plasmid** cer site and at least four chromosome-encoded proteins: the XerC and XerD recombinases, and accessory factors ArgR and PepA. It has been suggested that ArgR has a role in the initial pairing of **recombination** sites and we describe here an attempt to detect this process in vivo. Our approach exploits a previous observation that a cer-like site known as the type II **hybrid** supports inter-molecular **recombination** and causes extensive multimerization of plasmids. We report that type-II-mediated multimerization can be repressed by a cer site in cis or in trans and propose that this is due to a physical interaction between the sites. If

this hypothesis is correct, suppression of multimer formation provides an assay of site pairing. Our results demonstrate that the putative pairing interaction is independent of the topological relationship of the sites and that both PepA and ArgR are involved. Although most **recombination**-deficient mutant derivatives of ArgR are unable to pair **recombination** sites, we have found two (ArgR110 and ArgR115(*)) which retain pairing activity. The validity of the pairing hypothesis is discussed in the light of alternative explanations for our data.

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ACCESSION NUMBER: 95060450 EMBASE
DOCUMENT NUMBER: 1995060450
TITLE: The actinophage RP3 DNA integrates site-specifically into the putative tRNA(Arg)(AGG) gene of Streptomyces rimosus.
AUTHOR: Gabriel K.; Schmid H.; Schmidt U.; Rausch H.
CORPORATE SOURCE: Inst Genetik Mikrobiol Univ Munchen, Maria-Ward-Strasse 1A,80638 Munchen, Germany
SOURCE: Nucleic Acids Research, (1995) 23/1 (58-63). ISSN: 0305-1048 CODEN: NARHAD
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The temperate actinophage RP3 integrates site-specifically into the chromosome of Streptomyces rimosus R6-554. The phage attachment site **attP** and the **hybrid** attachment sites of the integrated prophage-attL and **attR**-were cloned and sequenced. The 54nt core sequence, common to all RP3 related attachment sites, comprises the 3' terminal end of a putative tRNA(Arg)(AGG) gene. **AttB** bears the complete tRNA gene which is restored in affL after integration. A 7.5 kb HindIII fragment, bearing **attP**, was used to construct an integrative **plasmid** to simulate the integration process in vivo and to localize the phage genes necessary for site specific integration. The int and xis genes were sequenced and compared to other **recombination** genes.

L16 ANSWER 16 OF 22 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 94107339 EMBASE
DOCUMENT NUMBER: 1994107339
TITLE: A DNA construct useful for specific chromosome loss in Saccharomyces cerevisiae.
AUTHOR: Kawasaki H.; Ouchi K.
CORPORATE SOURCE: Tokyo Research Laboratories, Kyowa Hakko Kogyo Co. Ltd., 3-6-6 Asahimachi,Machidashi, Tokyo 194, Japan
SOURCE: Journal of Fermentation and Bioengineering, (1994) 77/2 (125-130). ISSN: 0922-338X CODEN: JFBIEX
COUNTRY: Japan
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A GAL1-R/RS-RS cassette DNA was constructed to induce the loss of a specific chromosome in Saccharomyces cerevisiae. The cassette is composed of two specific **recombination** sites (RS) derived from the pSR1 **plasmid** of Zygosaccharomyces rouxii, and the site-specific **recombinase** gene, R, placed downstream of the GAL1 promoter. To delete a certain chromosome, the cassette was inserted into a site on that chromosome in a diploid cell by homologous **recombination** between

a DNA fragment cloned on the cassette and the relevant site on the chromosome. When the transformant was cultivated in galactose medium, elimination of the target chromosome occurred by R-promoted site- specific **recombination** between two unequal RS sites. Using this method, we demonstrated the loss of chromosomes III, V, VII and XV in heterozygous diploids. Aneuploids appeared at a frequency of 45-85% in the colonies examined after the induction of chromosome loss and were easily distinguished since they gave rise to smaller colonies than did the diploids. We confirmed that the aneuploids often duplicated the residual monosomic chromosome to restore the chromosome balance during mitotic growth. Such diploids formed colonies that were as large as the parent. This method is useful for conversion of heterozygous chromosomes into homozygous (or uniparental) ones in **hybrid** strains, and is also useful for chromosome loss mapping.

L16 ANSWER 17 OF 22 EMBASE
on STN

ACCESSION NUMBER: 93310911 EMBASE
DOCUMENT NUMBER: 1993310911
TITLE: High-level heterologous gene expression in Saccharomyces cerevisiae from a stable 2μm plasmid system.
AUTHOR: Ludwig D.L.; Ugolini S.; Bruschi C.V.
CORPORATE SOURCE: Microbiology Department, Internat. Ct. Gen. Eng./Biotechnol., Padriciano 99,I-34012 Trieste, Italy
SOURCE: Gene, (1993) 132/1 (33-40).
ISSN: 0378-1119 CODEN: GENED6
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The best candidate for a high-copy-number and mitotic stability expression system in yeast is the endogenous 2μm **plasmid**. Nevertheless, derivatives of the 2μm **plasmid** typically exhibit lower copy numbers and require selection for adequate maintenance within cells. We report the construction and utilization of an efficient heterologous gene expression system containing a 4.5-kb inducible expression cassette inserted into the 2μm **plasmid** and selected in cells utilizing a carrier **plasmid** which is subsequently lost via **FRT** /Flp **recombination**. The non-selectable 2μm **plasmid**, containing the cassette, was found to be stably maintained in cells, without selection, at high copy number. The dynamics of resolution and partitioning of this **plasmid** were analyzed during the course of 50 generations of growth under non-selective conditions. The heterologous lacZ reporter gene coding for β-galactosidase (βGal) is driven by the **hybrid**, galactose-inducible promoter GAL10::pMFα1. Upon induction, βGal was secreted into the periplasm and culture supernatant at levels which could be detected directly from Coomassie blue-stained SDS-PAGE. Furthermore, **plasmid**-containing cells could be maintained directly on rich YPD medium and identified either by utilizing XGal or by observing inhibition of colony growth on YPGal solid medium. The cassette was designed for direct, high-level, inducible expression of cloned genes downstream from the MFα1 signal sequence, with or without a C-terminal lacZ fusion. This **vector** represents the first demonstration of a non-selectable, mitotically stable, episomal **plasmid** system capable of expressing recombinant proteins at high levels. By supplanting the need for synthetic medium, this system could provide both an efficient and cost-effective means of generating recombinant protein at either the laboratory or large-scale level.

L16 ANSWER 18 OF 22 EMBASE
on STN

ACCESSION NUMBER: 93301579 EMBASE

DOCUMENT NUMBER: 1993301579
TITLE: Manipulation of transgenes by site-specific **recombination**: Use of Cre **recombinase**.
AUTHOR: Sauer B.
SOURCE: Methods in Enzymology, (1993) 225/- (890-900).
ISSN: 0076-6879 CODEN: MENZAU
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 021 Developmental Biology and Teratology
022 Human Genetics
LANGUAGE: English

L16 ANSWER 19 OF 22 EMBASE
on STN
ACCESSION NUMBER: 92132496 EMBASE
DOCUMENT NUMBER: 1992132496
TITLE: A phage T4 in vitro packaging system for cloning long DNA molecules.
AUTHOR: Rao V.B.; Thaker V.; Black L.W.
CORPORATE SOURCE: Department of Biology, 103 McCort Ward Hall, Catholic University of America, 620 Michigan Avenue, N.E.,Washington, DC 20064, United States
SOURCE: Gene, (1992) 113/1 (25-33).
ISSN: 0378-1119 CODEN: GENED6
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Recombinant **plasmid** DNAs containing long DNA inserts that can be propagated in Escherichia coli would be useful in the analysis of complex genomes. We tested a bacteriophage T4 in vitro DNA packaging system that has the capacity to package about 170 kb of DNA into its capsid for cloning long DNA fragments. We first asked whether the T4 in vitro system can package foreign DNA such as concatemerized λimm434 DNA and phage P1-pBR322 **hybrid** DNA. The data suggest that the T4 system can package foreign DNA as efficiently as the mature phage T4 DNA. We then tested the system for its ability to clone foreign DNA fragments using the P1-pBR322 **hybrid** vectors constructed by Sternberg [Proc. Natl. Acad. Sci. USA 87 (1990) 103-107]. E. coli genomic DNA fragments were ligated with the P1 vectors containing two directly oriented **loxP** sites, and the ligated DNA was packaged by the T4 in vitro system. The packaged DNA was then transduced into E. coli expressing the phage P1 cyclization **recombination** protein **recombinase** to circularize the DNA by **recombination** between the **loxP** sites situated at the ends of the transduced DNA molecule. Clones with long DNA inserts were obtained by using this approach, and these were maintained as single-copy plasmids under the control of the P1 **plasmid** replicon. Clones with up to about 122-kb size inserts were recovered using this approach.

L16 ANSWER 20 OF 22 EMBASE
on STN
ACCESSION NUMBER: 87058717 EMBASE
DOCUMENT NUMBER: 1987058717
TITLE: In vitro packaging into phage T4 particles and specific recircularization of phage lambda DNAs.
AUTHOR: Black L.W.
CORPORATE SOURCE: Department of Biochemistry, University of Maryland Medical School, Baltimore, MD 21201, United States
SOURCE: Gene, (1986) 46/1 (97-101).
CODEN: GENED6

COUNTRY: Netherlands
DOCUMENT TYPE: Journal
FILE SEGMENT: 004 Microbiology
022 Human Genetics
047 Virology
LANGUAGE: English
AB Concatemeric phage λimm434 DNA packaged in vitro into phage T4 particles produced plaques on a selective host. Moreover, λ DNA containing a pBR322 derivative flanked by the λ attL and **attR** sites could be specifically recircularized by excisive λ **recombination** to yield the pBR322 derivative. A host deficient in generalized **recombination** and containing a defective λcIts prophage which provided Int and Xis proteins was the recipient for this **plasmid** derivative carried by T4. Such a T4-λ **hybrid** may potentially allow almost one T4 headful of donor DNA (166 kb) to be packaged and recircularized.

L16 ANSWER 21 OF 22 EMBASE
on STN
ACCESSION NUMBER: 81217092 EMBASE
DOCUMENT NUMBER: 1981217092
TITLE: Denaturation map of bacteriophage P1 DNA.
AUTHOR: Meyer J.; Stalhammar-Carlemalm M.; Iida S.
CORPORATE SOURCE: Dept. Microbiol., Biozent., Univ., CH 4056 Basel, Switzerland
SOURCE: Virology, (1981) 110/1 (167-175).
CODEN: VIRLAX
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 047 Virology
022 Human Genetics
004 Microbiology
LANGUAGE: English
AB Electron microscopy of bacteriophage P1 **plasmid** DNA partially denatured by high pH in the presence of formaldehyde and mounted by the protein monolayer technique revealed a unique denaturation pattern. Alignment with the restriction cleavage map was obtained by comparing the melting pattern of **plasmid** DNA linearized by a single cut introduced by the restriction enzyme PstI with those of the large HindIII, BglII, and BamHI restriction fragments. Major A + T-rich segments center around map units 3, 21, 46, and 52. Other denatured regions are located around map units 29, 32 (within the invertible segment), 62, 88, and 96. The invertible segment of P1 DNA was precisely mapped within the Bam HI-5 and the BglII-5 DNA restriction fragments. The DNA segment melting first and therefore having the highest A + T content spans the P1-specific restriction modification region between map units 0 and 6 of the P1 genome. The P1-specific **recombination** site, **loxP**, is also located in a region of low DNA helix stability. The denaturation patterns of the DNA of a **hybrid** P1-R **plasmid** revealed IS1-mediated transposition of the r-determinant into a site adjacent to the most A + T-rich region on P1.

L16 ANSWER 22 OF 22 BIOSIS on STN
ACCESSION NUMBER: 1982:208124 BIOSIS
DOCUMENT NUMBER: PREV198273068108; BA73:68108
TITLE: BACTERIO PHAGE P-1 SITE SPECIFIC **RECOMBINATION** 2. **RECOMBINATION** BETWEEN **LOX**-P AND THE BACTERIAL CHROMOSOME.
AUTHOR(S): STERNBERG N [Reprint author]; HAMILTON D; HOESS R
CORPORATE SOURCE: CANCER BIOL PROGRAM, NATIONAL CANCER INST, FREDERICK CANCER RES CENT, FREDRICK, MD 21701, USA
SOURCE: Journal of Molecular Biology, (1981) Vol. 150, No. 4, pp. 487-508.

CODEN: JMOBAK. ISSN: 0022-2836.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB The events associated with **recombination** between **loxP**, a site in phage P1 EcoRI fragment 7, and loxB, a site in the bacterial [Escherichia coli] chromosome, are described. When fragment 7 is in a phage λ **vector**, the product of **loxP** + loxB **recombination** is the integration of the λ-P1:7(**loxP**) DNA as prophage in the bacterial chromosome and the production of 2 new **hybrid** sites, loxR and loxL, flanking the prophage. Lambda phages containing loxL, loxR and loxB sites were isolated and used to measure **recombination** frequencies between all 4 **lox** sites in paired phage crosses. **loxP** and loxL sites were much better substrates for **recombination** than are loxR and loxB sites. Both partners in a **recombination** reaction must contain either **loxP** or loxL sites for **recombination** to be efficient. These results can explain all of these findings relating to the **recombination** between λ-P1:7 DNA and the bacterial chromosome. In particular, integration of that DNA into loxB is low because **loxP** + loxB **recombination** is inefficient, and excision of the resulting prophage DNA is low because loxL + loxR **recombination** is inefficient. In contrast, integration of λ-P1:7 DNA into a chromosome already containing a i-P1:7 prophage at loxB is relatively high and is always accompanied by the excision and subsequent loss of the original prophage DNA. This process was called integration by prophage displacement, and it can be accounted for by postulating that 2 **lox recombination** events are involved (**loxP** + loxR and loxL), both of which are more efficient than is **loxP** + loxB **recombination**. Analysis of the physical structure of the various λ **lox** phages indicates that **loxP**-containing DNA can integrate into loxB in both possible orientations, and that the **loxP** + loxB crossover point is located within a 100 base-pair region of P1 BamHI fragment 9, a subfragment of EcoRI fragment 7. The loxB site was mapped to a region of the E. coli chromosome between tolC (66 min) and dnaG (67 min).